

AMENDMENTS

In the specification

Please insert the following paragraph on page 1 between lines 1-2

This application is a continuation of pending U.S. Application Serial No. 10/227,738 filed August 26, 2003, which is a continuation of 09/663,147 filed September 15, 2000, now abandoned, which is a continuation of U.S. Patent Application Serial No. 08/800,593 filed February 18, 1997, now U.S. Patent No. 6,143,505, which is a divisional of U.S. Patent Application Serial No. 08/317,450 filed October 4, 1994, now U.S. Patent No. 5,660,982.

Please replace the paragraph on page 1 lines 12-25 with the following:

Kalinin/laminin-5 (most likely also identical to the adhesion molecule nicein) is a recently identified laminin isoform which is a functional adhesion component for epithelial cells (Tryggvason, 1993, supra; Burgeson, et al., 1994, supra; Rousselle, et al., J. Cell. Bio., 1991, **114**: 567-576; Kallunki, et al., J. Cell Biol., 1992, **119**: 679-693; Marinkovich, et al., J. Biol. Chem., 1992, **267**: 17900-17906; Vailly, et al., Eur. J. Biochem., 1994, **219**: 209218).

Kalinin/laminin-5 contains unique laminin **variant** [~~variant~~] chains, one of which, the γ 2 chain, has been cloned and sequenced (Kallunki, et al., 1992, supra, previously named B2t). The γ 2 chain has a mass of \sim 130 kd and is thus smaller than the "classical" \sim 200 kd β 1 and γ 1 light chains of laminin. The domain structure of the γ 2 chain also differs from that of the γ 1 chain in that it lacks the amino-terminal globular domain (domain VI) believed to function in intermolecular cross-linking of laminin molecules to form networks (Yurchenco and O'Rear, in Molecular and Cellular Aspects of Basement Membranes, 1993, (Ed. Rohrbach and Timpl, Academic Press, San Diego, pp. 20-47). In addition, domains III, IV, and V (containing EGF-like repeats) in γ 2 are shorter than in the γ 1 chain (Kallunki, et al., 1992, supra).

Please replace the paragraph on page 3 lines 23-25 with the following:

Figure **2A-G'** shows In situ hybridization for γ 2 chain mRNA on sections of ductal mammary carcinoma (**2A-2A'**), malignant melanoma (**2B-B'**), squamous cell carcinoma of the skin (**2C-C'**),

Figure 2A-G' shows In situ hybridization for $\gamma 2$ chain mRNA on sections of ductal mammary carcinoma (2A-2A'), malignant melanoma (2B-B'), squamous cell carcinoma of the skin (2C-C', 2D-D'), and squamous cell carcinoma of the vulva (2E-E' and 2G-G'). Magnification: 2C-C' x 100, all others x 640.

Please replace the paragraph on page 3 lines 27-29 with the following:

Figure 3A-A' is incisionally wounded mouse skin (72 hours after wounding) showing signal for $\gamma 2$ chain in keratinocytes at the leading edge of the migrating epithelium (curved arrow). Magnification: x 640.

Please replace the paragraph on page 3 lines 31-36 with the following:

Figure 4 shows the nucleic [~~nucleic~~] acid sequence for the $\gamma 2$ chain cDNA and the derived amino acid sequence. Figure 4A is the full cDNA for the 5,200 base pair sequence, available from EMB/GenBank/DDBJ under the accession number Z15008. Figure 4B is the nucleotide and derived amino acid sequence of the alternative 3' end sequence from cDNA clones providing a sequence of 4,316 base pairs, available from EMB/GenBank/DDBJ under the accession number Z15009. (Kallunki, et al., 1992, supra.)

Please replace the paragraphs on page 7 line 34 to page 8 line 8 with the following:

DNA isolated either from fibroblast cultures or from specimens obtained from buccal smears, was used as template for amplification of genomic sequences. For amplification of introns 8 and 16, ~500 ng of genomic DNA was used as template and the following oligomer primers were utilized.

5' GGCTCACCAAGACTTACACA 3' (SEQ ID NO: 1);

5' GAATCACTGAGCAGCTGAAC 3' (SEQ ID NO: 2);

5' CAGTACCAGAACCGAGTTCG 3' (SEQ ID NO: 3);

5' CTGGTTACCAGGCTTGAGAG 3' (SEQ ID NO: 4);

5' TTACTGCGGAATCTCACAGC 3' (SEQ ID NO: 5);

5' TACACTGTTCAACCCAGGGT 3' (SEQ ID NO: 6);
5' AAACAAGCCCTCTCACTGGT 3' (SEQ ID NO: 7);
5' GCGGAGACTGTGCTGATAAG 3' (SEQ ID NO: 8);
5' CATACCTCTCTACATGGCAT 3' (SEQ ID NO: 9);
5' AGTCTCGCTGAATCTCTCTT 3' (SEQ ID NO: 10);
5' TTACAACTAGCATGGTGCCC 3' (SEQ ID NO: 11);

Please replace the paragraphs on page 10 lines 21-33 with the following:

RT-PCR and heteroduplex analysis (MDE)

50 µg of total RNA isolated from cultured keratinocytes from JEB patient, and unrelated healthy controls were reverse transcribed in a volume of 100 µL as recommended by the manufacturer (BRL). 1 µL of the reaction product was used to amplify overlapping regions of the cDNA that spanned the open reading frame. Primer pair used to identify the mutation R95X: (L) 5'-GAGCGCAGAGTGAGAACCAC-3' SEQ ID NO: 16, (R) 5'-ACTGTATTCTGCAGAGCTGC-3' SEQ ID NO: 17. PCR cycling conditions were: 94 C, 5 min, followed by 94 C, 45 sec; 60 C, 45 sec; 72 C, 45 sec; for 35 cycles, and extension at 72 C for 5 min. 5 µL aliquots were run in 2% agarose gels. Heteroduplex analysis was performed as recommended by the manufacturer (MDE, AT Biochemicals). Heteroduplexes were visualized under UV light in the presence of ethidium bromide and photographed. Amplified cDNA fragments with altered mobility were subcloned into the TA vector according to the manufacturer's recommendations (Invitrogen). Sequence analyses were then performed using standard techniques.

Please replace the paragraph on page 13 lines 29 to page 14 line 4 with the following:

Results: Laminin α 1, β 1, γ 1, and γ 2 chains

All rounds of *in situ* [~~situ~~] hybridization include both sense and anti-sense RNA probes for each of the genes studied. As negative controls, sense RNA probes are applied to adjacent sections

and these probes consistently are negative. As a positive control of the $\gamma 2$ chain hybridizations, two anti-sense probes derived from non-overlapping $\gamma 2$ chain cDNA clones are used on a number of sections. To summarize [summarizes] the $\gamma 2$ chain expression found; all carcinomas were positive except for one case of mammary duct carcinoma, and all three cases of leiomyosarcomas, both cases of malignant fibrous histiocytoma, and the only case of neurofibrosarcoma. The positive controls always gave similar staining on adjacent sections (see Figure 2, E and G). Fifteen of the malignant cases and all mouse tissue blocks were hybridized on two or more separate occasions giving the same hybridization pattern. All cell types other than those described below were negative in all cases.